controls. Histological examination of the gonads confirmed the presence of very abundant lutein tissue and demonstrated the formation of this tissue about the egg in unruptured, normal follicles and in atretic follicles. Ripe, normal Graafial follicles were invariably absent. *A powerful, specific stimulus to lutein cell transformation has thus been effected by this hormone.*

Aside from the fact that it is tolerated only in smaller quantities, fresh posterior hypophyseal substance gave none of the above characteristic effects upon growth, oestrus and ovulation in animals as compared with similar care with litter mate sisters.

* Aided by grants from the Committee on Scientific Research of the American Medical Association.

A STUDY OF THE EFFECTS OF CUCURBITA PEPO SEEDS ON KIDNEY EXCRETION

BY BENJAMIN MASUROVSKY

FROM PHYSIOLOGICAL CHEMISTRY LABORATORY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY

Communicated by R. Pearl, February 4, 1922

1. *Purpose and Plan of Experiment.*—The object of this experiment was to compare and verify the results obtained from a somewhat similar experiment conducted during March 1920.[1] Moreover, a further study of the effects of Cucurbita seeds on kidney excretion is thereby promulgated.

Since the previous experiment does not give sufficient analytical data so far as the urine samples are concerned a more elaborate plan was followed by virtue of which not only comparative data may be obtained but additional analyses may be available for the elucidation of the problem under consideration.

Three successive dietary periods were arranged for the experiment, namely, three days of a preliminary basal dietary period, three days of an experimental dietary period, and three days of a final dietary period. The entire experiment lasted for nine consecutive days.

All the foodstuffs consumed in this experiment were measured. The pumpkin seeds (Cucurbita pepo) were of two kinds, roasted and raw, a known quantity of each kind was added to each meal during the experimental dietary period.

2. *Dietary.*—Hindhede's "back-to-the-farm" dietary,[2] slightly modified, was used for the basal diet. Composition of this dietary is given in table 1. This diet was used during the preliminary and final periods of this experiment. The experimental diet consisted of the same basal diet plus 30 grams of roasted pumpkin seeds for the first day; 35 grams of roasted and 30 grams of raw pumpkin seeds mixed for the second day, and 30 grams of raw seeds for the third day of the experimental dietary period.

TABLE 1

COMPOSITION OF THE BASAL DIETARY[3]

NAME	QUANTITY	PROTEIN	FAT	CARBOHYDRATES	CALORIES
Water	600 cc.				
Graham bread	300 gms.	19.7	4.8	153.9	780
Apples	600 gms.	1.8	3.0	76.8	342
Milk	800 cc.	25.6	30.4	40.0	620
Butter	60 gms.	.6	48.4	...	450
Potatoes	800 gms.	15.2	.8	160.0	800
Total		62.9	87.4	430.7	2992

No salt was used.

3. *Period I.*—In securing the daily samples of urine the bladder was emptied about 7 a. m., April 3rd, and this urine was discarded. All the urine from that hour up to and including that passed the next day at 7 a. m. was saved, thoroughly mixed, preserved with 5 cc. of toluene, a sufficient amount to film the bottom and the walls of the container. The latter were wide mouth glass jars with ground glass stoppers. Thus, each composite sample represented the mixed excretions for twenty-four hours.

As soon as a twenty-four hour sample was secured it was immediately analyzed for the determination of volume, specific gravity, temperature, titratable acidity (using 0.1 *N* NaOH) (4, pp. 508–509), NH_3 determination (Folin's method) (4, p. 523); total nitrogen determination (Kjeldahl method) (4, pp. 512–513); creatinine (Folin's colorimetric method) (4, pp. 530–531); urea (Marshall's method) (4, p. 522); uric acid (Folin and Schaffer method) (4, p. 536); and total solids of a sample were computed by means of Long's coefficient (4, p. 512). The remainder of each of the samples was then covered with a layer of toluene to preserve it for the inorganic analyses.

TABLE 2*a*

ANALYSES OF COMPOSITE SAMPLES OF URINE FROM PERIOD I

DATE 1921	VOLUME CC.	TEMP. C.	SP. GR.	T. S. GMS.	ACIDITY CC. 0.1 *N* NaOH PER 100 CC.	TOTAL N GMS.
Apr. 3	1360	24	1.019	65.77	39.0	11.72
Apr. 4	1600	22	1.017	66.56	24.3	9.85
Apr. 5	1710	24	1.016	69.35	10.8	10.04

TABLE 2*b*

NITROGENOUS INGREDIENTS FOUND IN SAMPLES OF URINE, PERIOD I

DATE 1921	AMMONIA	CREATININE	URIC ACID	UREA	UNDETERM. N
Apr. 3	.38	1.07	.57	20.28	1.37
Apr. 4	.48	1.24	.65	17.17	.78
Apr. 5	.31	1.28	.73	18.54	.39

4. *Period II.*—The pumpkin seeds used during this experiment were obtained from a seed grower. The roasting was done after the hulls were removed. The hull-free seeds were placed in a flat glass dish and kept in a dry-heat oven for three hours at a temperature of 110° F. or 43° C.

The table below shows the quantities of pumpkin seeds ingested at each meal during the experimental Period II.

KIND	APRIL 6 BREAKF. GMS.	APRIL 6 LUNCH GMS.	APRIL 6 DIN. GMS.	APRIL 7 BREAKF. GMS.	APRIL 7 LUNCH GMS.	APRIL 7 DIN. GMS.	APRIL 8 BREAKF. GMS.	APRIL 8 LUNCH GMS.	APRIL 8 DIN. GMS.
Roasted	5	10	15	15	15	5	..	..	.
Raw p. s.	.	..	..	5	10	15	15	10	5

The same methods of sampling and analyzing employed in Period I were also used in Period II of this experiment.

TABLE 3*a*

ANALYSES OF COMPOSITE SAMPLES OF URINE FROM PERIOD II

DATE 1921	VOLUME CC.	TEMP. C.	SP. GR.	T. S. GMS.	ACIDITY CC. 0.1 *N* NaOH PER 100 CC.	TOTAL N GMS.
Apr. 6	1425	20	1.019	64.46	17.8	9.69
Apr. 7	1945	19	1.013	55.62	15.1	10.14
Apr. 8	2150	22	1.014	72.67	14.2	12.08

TABLE 3*b*

NITROGENOUS INGREDIENTS FOUND IN SAMPLES OF URINE FROM PERIOD II

DATE 1921	AMMONIA	CREATININE	URIC ACID	UREA	UNDETERM. N
Apr. 6	.41	.79	.74	17.69	.58
Apr. 7	.44	1.01	.79	17.88	.76
Apr. 8	.56	1.34	.85	20.61	1.19

5. *Period III.*—During the final three days, April 9, 10, 11, of this experiment the diet was exactly the same as during the preliminary Period I.

TABLE 4*a*

ANALYSES OF COMPOSITE SAMPLES OF URINE FROM PERIOD III

DATE 1921	VOLUME CC.	TEMP C.	SP GR	T. S. GMS.	ACIDITY CC. 0.1 *N* NaOH PER 100 CC.	TOTAL N GMS.
Apr. 9	1705	22	1.016	66.49	20.5	10.16
Apr. 10	2370	17	1.015	76.40	7.5	9.70
Apr. 11	1910	18	1.016	67.53	12.8	9.45

TABLE 4*b*

NITROGENOUS INGREDIENTS FOUND IN SAMPLES OF URINE, PERIOD III

DATE 1921	AMMONIA	CREATININE	URIC ACID	UREA	UNDETERM. N
Apr. 9	.53	1.18	.62	16.58	1.37
Apr. 10	.55	1.35	.63	17.26	.49
Apr. 11	.50	1.32	.66	15.63	1.04

6. *Discussion.*—To reduce the effect of the variation factor upon the study of results and to obtain a more satisfactory comparison the hypothetical mean of results of each period was computed.

The following table V presents the data for comparison.

TABLE 5

COMPARATIVE RESULTS FOUND DURING PERIODS I, II AND III
(HYPOTHETICAL MEANS)

PERIOD	VOLUME CC.	T. S. GMS.	ACIDITY CC. 0.1 *N* NaOH	T. N. GMS.	NH_3 GMS.	CREATININE GMS.	URIC ACID, GMS.	UREA GMS.
I	1655	67.95	17.5	9.94	.39	1.26	.69	17.85
II	2047	64.14	14.6	11.11	.50	1.17	.82	19.24
III	2140	71.96	10.15	9.57	.52	1.33	.64	16.44

From this table we find that the average daily output of urine during Period II was greater than that of Period I but smaller when compared with the average daily volume of urine excreted during Period III.*

The amount of total solids of the experimental Period II was less as compared with either of the basal periods (I and II) which served as a check in this work. The acidity of the urine excreted during Period II was on the average slightly greater than that of Period III which agrees with the results obtained in the first experiment.[1]

The total nitrogen output is highest in Period II due to the fact that additional feeding substances, in the form of pumpkin seeds, were added to the basal diet.

Of the nitrogenous ingredients of the average samples of the three periods, uric acid of Period II shows a marked relation to that of Periods I and III. Namely, taking the average quantity of uric acid excreted during Period III as 100%, we find that the average output of uric acid during Period II was 128.12%.

Creatinine of the average sample of urine excreted during Period II as compared to that of Period III is as 100% is to 87.97%. The excretion of urea during Period II was 117%.

A comparative qualitative test (Obermayer's) for indican of all the nine samples of urine gave the results shown in table 6 below:

TABLE 6

COMPARATIVE INDICAN CONTENT

SAMPLE NO.	GRAPHIC REPRESENTATION	RANK ACCORDING TO INTENSITY OF COLOR
1	******	4th place
2	***	7th place
3	**	8th place
4	****	6th place
5	*******	3rd place
6	*********	1st place
7	********	2nd place
8	*****	5th place
9	*	9th place

It is of significant interest to note that the last two samples of the experimental period and the first sample of the final period gave the deepest coloration when tested for indican.

7. *Inorganic Determinations.*—For the determination of total sulphates and ethereal sulphates Folin's methods were employed; total phosphates were determined by the Uranium Acetate method; for the determination of chlorides and chlorin Volhard-Arnold method was employed.

TABLE 7

MINERAL INGREDIENTS FOUND IN THE URINE SAMPLES, GMS.

SAMPLE NO.	CHLORIDES	Cl	TOTAL P_2O_5	TOTAL SO_3	ETHEREAL SO_3
1	9.05	5.43	1.67	1.57	.268
2	8.66	5.20	1.56	1.60	.367
3	7.42	4.45	1.69	1.64	.328
4.6	6.61	3.96	1.71	1.76	.332
5	6.51	3.90	1.87	1.78	.346
6	7.86	4.72	2.08	1.97	.426
7	6.83	4.10	1.73	1.70	.331
8	7.77	4.66	3.14	1.83	.299
9	7.28	4.39	1.74	1.83	.288

8. *Conclusions.*—I. The results obtained in the experiment substantiate the results of the first experiment inasmuch as the volume and total solids output during the experimental Period II was less than in the check Period III; the hypothetical volume for the check period being 2140 cc. while during the experimental Period II the output was 2047 cc.; the average output of total solids during the check period being 71.96 while during the experimental Period II it was 64.14 gms.

II. There was 28.12% more uric acid eliminated during Period II than during the check Period III.

III. Also 17% more urea was eliminated during the experimental period than during the check Period III.

IV. The striking difference in the intensity of color in the urine samples of the experimental period revealed by the indican test is of pathological significance.

V. From the verified results and additional data obtained in this experiment the rôle played by Cucurbita pepo seeds in animal metabolism is of a chemico-pharmacognostic value.

I wish to express my thanks to Prof. W. J. Gies (Columbia) and Dr. E. G. Miller, Jr. (Columbia), for helpful suggestions in this work and to Dr. J. G. Lipman, Director of N. J. Agr. Exp. Station, for material assistance with this experiment.

* For the sake of uniformity in this work the mean of only the second and third days' results of each period was taken. For it is evident that the first day's excretions are only slightly affected by the change of diet.

[1] Masurovsky, B., "Effects of Some Cucurbita Seeds on Animal Metabolism," *J. Agric. Res.*, **21**, No. 8, 1921 (523).

[2] Lusk, G., *Science of Nutrition*, p. 340.

[3] Atwater and Bryant, "The Chemical Composition of American Food Materials," *Bull.* **28** (Revised), *U. S. Dept. of Agriculture*, 1902.

[4] Hawk, P. B., *Practical Physiological Chemistry*, 6th edition, 7 pl. (col.), 1920.